Original Article

Effects of Solvents on the In-vitro Antioxidant Activity of Dennettia tripetala G. Baker and Milicia excelsa (Welw.) C. Berg Root Extracts

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Abstract

***Background and Purpose*:** The root extracts of Dennettia tripetala G. Baker and Milicia excelsa (Welw.) C. Berg was investigated for their in-vitro antioxidant activities.

***Materials and Methods*:** The pulverized roots of Dennettia tripetala and Milicia excelsa were extracted separately by cold maceration using ethyl acetate, methanol, n-butanol and water respectively as the extracting solvents. In-vitro antioxidant, the activity of root extracts was then investigated using DPPH model. The results obtained was analyzed using One-way Analysis of Variance involving GraphPad Prism 5 Software.

***Results*:** The antioxidant assay of various extracts, using 2, 2-diphenyl-1-picrylhydrazyl radical scavenging model, revealed that the ethanol and n-butanol extracts of D. tripetala had better radical scavenging activity with IC_{50} values of 2.02 and 0.631 µg/mL for ethanol and n-butanol extracts, respectively. The methanol and ethanol extracts of M. excelsa showed higher radical scavenging capacity with IC50 of 0.194 and 8.84 µg/mL compared to that of the ascorbic acid which had IC50 value of 4.60 µg/mL.

***Conclusion*:** The radical scavenging ability of the extracts could be attributed to the presence of flavonoids and phenolics.

***Keywords*:** Phytochemicals; Antioxidant; Dennettia tripetala; Milicia excels; DPPH

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1. Introduction

Phytochemicals are plant metabolites that contain protective, disease-preventing and curative compounds which can lead to physiological changes (1). Plant-derived antioxidants are polyphenolic molecules which can inhibit the oxidative stress created by free radical *in vivo* (2). Free radical once formed can initiate a chain reaction capable of damaging vital molecules like lipids, proteins and nucleic acids in the body. Antioxidants interact with these free radicals before they cause oxidative damage to the body. Oxidative damage by free radical can lead to diseases like atherosclerosis, heart disease, diabetes mellitus, and cancer (3). Synthetic antioxidants like butylated hydroxylanisole (BHA), butyl hydroxytolune (BHT), α-tocopherol, and propyl gallate are commercially available but are limited in use due to their toxicity (4). Therefore, the search for natural antioxidants with high safety profile is recommended. *Dennettia tripetala*, commonly known as pepper fruit tree, is found mostly in tropical Africa and especially in southern, eastern and western Nigeria. The Igbo's call it "*nmimi*", while the Yoruba's call it "*igberi*". The fruits possess strong pepper-like and pungent spicy taste with a characteristic aroma and fragrance (5). In folk medicine, the leaves of *Dennettia tripetala* are used by herbalists in combination with other medicinal plants to treat various ailments including fever, convulsion, cough and stomach upset (6). *Milicia excelsa* is a deciduous forest tree of lowland forest and wet savannah. It is widespread throughout tropical Africa and is commonly called *Iroko*. The Igbo's of eastern Nigeria call it "*oji*", while the Hausas call it "*madachi*". The powdered bark of *Milicia excelsa* is used for management of cough, heart problems, and lassitude. The wound healing property of an ointment formulated using leave extract of *Milicia excelsa* in experimental animals has been reported (7). The antioxidant activity of *Dennettia tripetala* has been determined (8), but no report has so far been documented on the effect of solvent on the antioxidant of this plant. Thus, the present study involved the effect of solvent on the *in vitro* antioxidant activity from two Nigerian medicinal plants extracts.

2. Materials and Methods

2.1. Chemicals and reagents

Ethyl acetate, methanol, ethanol, *n*-butanol was bought from Sigma-Aldrich (Germany). 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ammonia solution, H_2SO_4, and $FeCl_3$ were also purchased from BDH (England). All chemicals used were of analytical grade. Distilled water was obtained from National Centre for Equipment Maintenance and Development (NCEMD), University of Nigeria, Nsukka.

2.2. Plant material

Fresh roots of *Dennettia tripetala* were collected from Nsukka, while the roots of *Milicia excelsa* were harvested from the University of Nigeria Botanical Garden in the month of June 2014 and were authenticated by Mr. A. O. Ozioko of International Center for Ethnomedicine and Drug Development (InterCEED), Nsukka. The voucher specimens of the plants were deposited at the herbarium of the botanical garden with voucher numbers ICEED/DT-009 and ICEED/ME-010, respectively.

2.3. Preparation of plant extracts

The air-dried roots were pulverized and the powdered material (10.0 g) were each macerated separately with 100 mL of ethyl acetate, methanol, ethanol, *n*-butanol and

water, and extracted at room temperature for 48 h with agitation. The filtrates were concentrated on *in vacuo* at reduced pressure and temperature (40°C) to obtain the dry extracts.

2.4. Qualitative Phytochemical analysis of the Extracts

The qualitative phytochemical analyses of the extracts were done to determine the presence of flavonoid, tannin and phenolics according to standard methods (9).

2.4.1. Test for flavonoids

0.50 g of the extracts was dissolved in 5 mL of distilled water and filtered. 5 mL of 10% (v/v), ammonia solution was added to the filtrate followed by three drops of concentrated H_2SO_4. Presence of flavonoids was confirmed by yellow coloration which disappears on addition of H_2SO_4.

2.4.2. Test for Tannins/Phenolics

0.50 g of the extracts was dissolved in 5 mL of distilled water and filtered. Two drops of 0.1% (v/v) $FeCl_3$ was added to the filtrate and observed for colour change. A blue-black colouration was taken as evidence for the presence of tannins or phenolics.

2.5. *In vitro* antioxidant assay

The antioxidant activity of the extracts was determined *in vitro* using the DPPH model as previously reported by Agbo *et al.* (10). Briefly, 100 mg of the extracts were dissolved in 100 mL of methanol to form stock solutions (1 mg/mL or 1000 μg/mL). Serial dilutions (10, 25, 50, 100, 250, and 500 μg/mL) of each extract were made from the stock solution. 3 mL of the DPPH solution (4.5 mg/100 mL of methanol) was added to 1.0 mL of the various concentrations of extracts was the mixture incubated at room temperature for 30 mins in the dark. The absorbance of the mixture and the standard (ascorbic acid) were determined at 517 nm against a blank with a UV/Visible spectrophotometer (UV-1800, Shimadzu, Japan). The assay was carried out in triplicate. The percentage inhibition was determined using the following formula:

$$\text{Percentage inhibition} = \frac{Ab - (As - Ac)}{Ab} \times 100$$

where, Ab = absorbance of the blank, As = absorbance of the sample, Ac = absorbance of the control.

2.6. Statistical analysis

The data were expressed as mean ± SEM of at least triplicate determinations (n = 3). To demonstrate the statistical significance of data, a One-way Analysis of Variance (ANOVA) using GraphPad Prism 5 Software was performed followed by Dunnett's *posthoc* test. The differences between test and control treatments were considered significant at $p < 0.05$.

3. Results

3.1. Extraction yield and phytochemical screening

Cold maceration of powdered roots of *Dennettia tripetala* and *Milicia excelsa* in different solvents yielded the ethyl acetate, methanol, ethanol, and *n*-butanol, and aqueous extracts. The extracts were screened for phytochemical that can scavenge free radicals (flavonoids, tannins/phenolics), as shown in Table 1.

Table 1. Qualitative Phytochemical Analysis of the Extracts

	Dennettia tripetala			*Milicia excelsa*		
Solvents	**Flavonoids**	**Phenolics**	**Tannins**	**Flavonoids**	**Phenolics**	**Tannins**
Ethyl acetate	+++	++	-	++	+	+
Methanol	+	+	-	++	++	++
Ethanol	+	+	-	+	+	+
***n*-Butanol**	+	+	-	+	+	+
Water	-	-	-	+	+	+

Key: +++ = high in abundance; ++ = moderate in abundance; + = low in abundance; - = absent

3.2. DPPH radical scavenging ability

The *in-vitro* antioxidant activities of the extracts were determined using the DPPH Model (Tables 2&3 and Figure 1).

Table 2. Effects of extracting solvents on the DPPH scavenging activity (%) of the various extracts of *D. tripetala*

	Concentration (mg/L)					
Extracts	**10**	**25**	**50**	**100**	**250**	**500**
EtOAc	48.87 ± 0.45	54.67 ± 0.41	58.15 ± 0.37	59.67 ± 0.33	74.87 ± 0.28	76.26 ± 0.34
MeOH	35.55 ± 0.20	39.57 ± 0.18*	41.20 ± 0.22	42.46 ± 0.32	53.39 ± 0.30	56.21 ± 0.12
EtOH	89.75 ± 0.61	90.88 ± 0.76	91.00 ± 0.69	92.26 ± 0.55*	93.00 ± 0.72	94.27 ± 0.68*
***n*-BuOH**	56.53 ± 0.49*	62.38 ± 0.54	62.94 ± 0.44	63.31 ± 0.40	65.82 ± 0.60	68.71 ± 0.56
Aqueous	39.07 ± 0.27	41.83 ± 0.32	49.37 ± 0.34	52.14 ± 0.25	54.27 ± 0.30*	56.88 ± 0.41
ASA	67.89 ± 0.30	89.00 ± 0.25	91.68 ± 0.21	94.63 ± 0.39	95.66 ± 0.41	96.87 ± 0.29

Results are expressed as mean ± SD (n=3), $p < 0.05$ significant. Dunnett's post-hoc LSD (least significant difference) test. *$p < 0.05$, comparison with ASA. The absorbance against the reagent blank was determined at 517 nm with a UV-Visible spectrometer; EtOAc = ethyl acetate extract, MeOH = methanol extract, EtOH = ethanol extract, *n*-BuOH = butanol extract, Aqueous = water extract, ASA = ascorbic acid.

Table 3. Effects of extracting solvents on the DPPH scavenging activity (%) of the various extracts of *M. excelsa*

	Concentration (mg/L)					
Extracts	**10**	**25**	**50**	**100**	**250**	**500**
EtOAc	54.55 ± 0.44	54.82 ± 0.42	62.56 ± 0.39	65.95 ± 0.54	77.40 ± 0.58	79.82 ± 0.60
MeOH	84.55 ± 0.67	90.83 ± 0.50	93.10 ± 0.63	94.35 ± 0.49*	95.59 ± 0.38	95.96 ± 0.28
EtOH	83.34 ± 0.70	86.10 ± 0.66	87.36 ± 0.78	87.62 ± 0.68	88.86 ± 0.47	90.01 ±0.74
***n*-BuOH**	33.29 ± 0.27	35.80 ± 0.30*	36.80 ± 0.40	38.06 ± 0.22	44.10 ± 0.40	47.24 ± 0.45
Aqueous	37.81 ± 0.23	43.47 ± 0.36	45.85 ± 0.55	50.51 ± 0.62	52.37 ± 0.23	53.13 ± 0.33*
ASA	67.89 ± 0.30	89.00 ± 0.25	91.68 ± 0.21	94.63 ± 0.39	95.66 ± 0.41	96.87 ± 0.29

Results are expressed as mean ± SD (n = 3), $p < 0.05$ significant. Dunnett's post-hoc LSD (least significant difference) test. *$p < 0.05$, comparison with ASA. The absorbance against the reagent blank was determined at 517 nm with a UV-Visible spectrometer; EtOAc = ethyl acetate extract, MeOH = methanol extract, EtOH = ethanol extract, *n*-BuOH = butanol extract, Aqueous = water extract, ASA = ascorbic acid

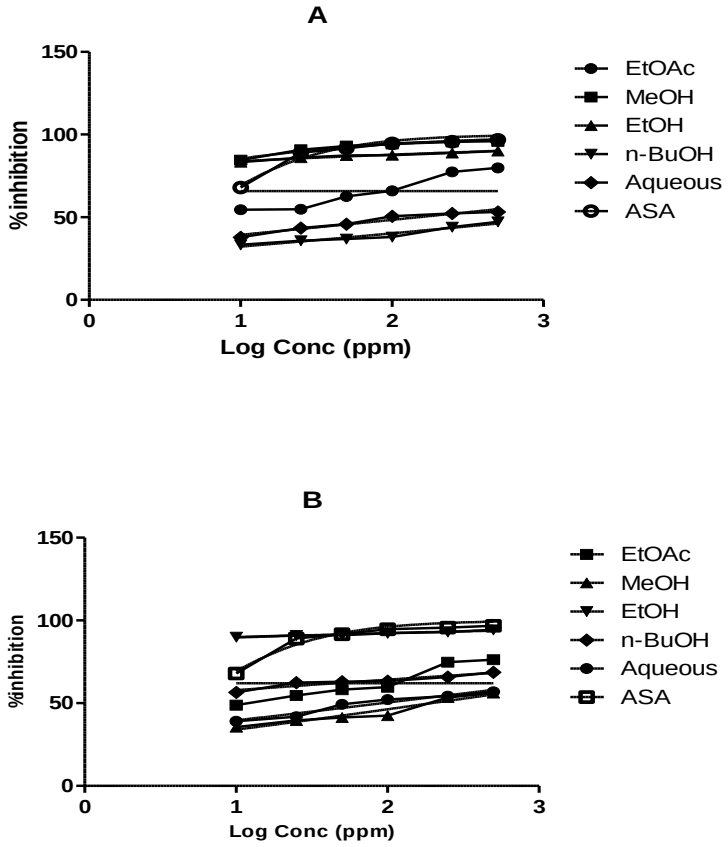

Figure 1. Scavenging activity on DPPH radicals (%) of the extracts (A = *D. tripetala*, B = *M. excels*a) obtained with ethyl acetate (EtOAc), methanol (MeOH), ethanol (EtOH), butanol (*n*-BuOH), water (Aqueous) and ascorbic acid (ASA).

The ethanol and *n*-butanol extracts of *D. tripetala* showed better radical scavenging activity than ascorbic acid. Also, the methanol and ethanol extracts of *M. excelsa* showed promising antioxidant ability than ascorbic acid.

Table 4. [a]IC_{50} values of the extracts of *D. tripetala* and *M. excelsa*

***D. tripetala* extracts**	**IC_{50} (µg/mL)**	**R^2**	***M. excelsa* extracts**	**IC_{50} (µg/mL)**	**R^2**
EtOAc	ND	-	EtOAc	ND	-
MeOH	194.0	0.9288	MeOH	0.1943	0.9563
EtOH	2.027	0.9423	EtOH	8.84	0.9592
***n*-BuOH**	0.6309	0.9094	*n*-BuOH	1382	0.9414
Aqueous	91.99	0.9443	Aqueous	148.8	0.9425
ASA	4.60	0.9474	ASA	4.60	0.9474

[a]IC_{50} value was calculated from the least squares regression equations in the plot of the % inhibition *vs*. logarithm of six graded concentrations. ND = Not detected; EtOAc = ethyl acetate extract, MeOH = methanol extract, EtOH = ethanol extract, *n*-BuOH = butanol extract, Aqueous = water extract, ASA = ascorbic acid.

4. Discussion

The extraction of phytochemicals from plant materials largely depends on the solvent of extraction (11). However, the antioxidant activities of the extracts are greatly dependent on the distribution of the phytochemicals in the extracts. The polarity of the extracting solvents used determine the yield and antioxidant activity of the plant extracts (12). The best extracting

solvent may not be determined easily due to the structure and composition of the secondary metabolites present. In the present study, different solvents (ethyl acetate, methanol, ethanol, *n*-butanol, and water) were used for the extraction of roots of *D. tripetala* and *M. excelsa* to ascertain the *in-vitro* antioxidant activities of the extracts. Polar secondary metabolites were extracted using polar solvents while the non-polar secondary metabolites were extracted using non-polar solvents. Flavonoids and phenolics were extracted in methanol, ethanol, and *n*-butanol from the *D. tripetala* root powder while flavonoids and tannins/phenolics were extracted with methanol, ethanol, *n*-butanol, and water in *M. excelsa* powder. This result agrees with previously reported work on the antioxidant phenolics from the methanol extracts of plant material (13). This is evident from the lower IC_{50} of these extracts indicating that the extracts had good radical scavenging activity. Numerous *in-vitro* models exist for the assay of the antioxidant capacity of plant extracts. DPPH assay remains the most acceptable model owing to its simplicity and reproducibility. This model involves the reduction of the DPPH radical by the antioxidant compounds which are evident from the colour change from purple to yellow (14). This loss in colour serves as the basis for the antioxidant assay which can be quantified spectrophotometrically at λ_{max} 517 nm. The DPPH radical scavenging ability of the root extracts of *Dennettia tripetala* is shown in Figure 3A. The radical scavenging capacity of the extracts were found to be 76.26%, 56.21%, 94.27%, 68/71%,56.88% under the concentration of 500 μg/mL for ethyl acetate, methanol, ethanol, *n*-butanol, and water, respectively (Table 2). The order of the DPPH scavenging activity of the extracts were ethanol extract > ethyl acetate > *n*-butanol > water > methanol. The high levels of polyphenolics like flavonoids in the ethyl acetate and ethanol root extracts of *D. tripetala* could be responsible for the high radical scavenging abilities of the extracts. This finding agrees with the work of Okolie *et al.* (15) that attributed the H_2O_2 scavenging activity of the extract of *D. tripetala* to the high levels of polyphenols in the extract. The changes in total phenol content and antioxidant activity of *Dennettia tripetala* with ripening have also been studied (16). The extracts of *M. excelsa* possessed concentration dependent antioxidant activity as evident from the varying degree of their radical scavenging properties ranging from 47.24 to 96.87% (Table 3) at a concentration of 500 mg/L. Maximum antioxidant activity was produced by the methanol extract (95.96%) followed by the ethanol extract (90.01%), while the least DPPH radical scavenging activity was offered by the *n*-butanol extract (47.24%). The 50% inhibitory concentrations of the methanol and ethanol extracts of *M. excelsa* was found to be 0.1943 and 8.840 μg/mL, respectively, compared to that of the standard (ascorbic acid) which was found to be 4.60 μg/mL (Table 4). In conclusion, the antioxidant effects of the extracts could be attributed to the polyphenolics/flavonoids in the extracts (Table 1). This agrees with the work of van der Sluis *et al.* (17) that showed high presence of these plant metabolites in the methanol and ethanol extracts of *M. excelsa* root.

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Conflicts of interest

We declare that there is no conflict of interest in this research. This research receives no funding/grant from either the public or non-profit organizations.

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